Effect of Fluorination on the Pharmacological Profile of 11β Isomers of Fulvestrant in Breast Carcinoma Cells

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We describe the synthesis of an 11β isomer **3** of the steroidal antiestrogen fulvestrant **2**. Partial fluorination of the 11β side chain in **3** leads to **4**, which still shows strong antiproliferative activity on MCF-7 cells. However, unlike **2** and **3**, compound **4** fails to down-regulate estrogen receptor α (ERα). This result suggests that ERα down-regulation is not a sine qua non condition for the antitumor activity of steroidal antiestrogens.

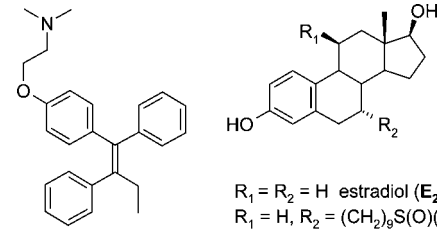
Introduction

The triphenylethylene-based antiestrogen tamoxifen (**1**) remains the gold standard for the treatment of estrogen receptor α (ERα)-positive breast cancer in premenopausal patients, in the adjuvant setting or as the first-line therapy of metastatic disease. Yet the untoward effects associated with the pharmacological profile of this drug (namely, its partial agonism) and the frequent occurrence of drug resistance have prompted the search for antiestrogens devoid of estrogenicity (i.e., "pure antiestrogens"). Several studies have shown that estradiol (E_2) derivatives bearing a functionalized side chain in position 7α satisfy this criterion. Among these compounds, fulvestrant (ICI 182,780, Chart 1) (**2**) was selected for clinical trials because of its high in vivo antitumor activity, notably in animal models of tamoxifen-resistant breast cancer,[1] and is currently in clinical use.

Structural studies based on X-ray crystallography data of the ligand binding domain (LBD) of the estrogen receptor bound to a steroidal antagonist (ICI 164,384 complexed with the LBD of ERβ) revealed that the terminal portion of its 7α side chain penetrates into a cleft located near the ligand binding pocket, thereby preventing the helix-12 repositioning associated with estrogenic stimulation.[2] Thus, the conformational change imposed by fulvestrant impedes the binding of coactivators harboring a LxxLL consensus motif (L = leucine, x = any amino acid). Notably, this step is required for the cascade of molecular events that leads to estrogenic responses. Hence, the absence of such coregulator recruitment after a treatment with fulvestrant partly explains its strong antiestrogenicity. The complementary ability of fulvestrant to desensitize breast tumor cells to estrogenic stimulation by inducing a rapid proteasomal degradation of ERα[3] has been reported to be another factor of major importance.[4,5]

When grafted onto E_2, 7α/11β alkyl side chains interact similarly with the surface of ERα protein, provided that they are not too large and/or too rigid to allow for the insertion and/or the rotation of the steroid nucleus within the ligand binding pocket.[6,7] The possibility that such a property also holds for the 7α side chain of fulvestrant led us to synthesize an 11β isomer **3** with the hope of producing a strong antiestrogen with a different endocrine/metabolic profile. Furthermore, our finding that perfluorination of a long 11β alkyl side chain may modify ligand interactions with the binding pocket[8] prompted us to synthesize a partially fluorinated derivative **4** of this 11β fulvestrant isomer. The present paper, which compares the biological properties of these two compounds (**3** and **4**) with those of fulvestrant (**2**), provides new insight in the mechanism of action of steroidal antiestrogens.

Chart 1. Estrogens and Antiestrogens



$R_1 = R_2 = H$ estradiol ($\mathbf{E_2}$)
$R_1 = H$, $R_2 = (CH_2)_9S(O)(CH_2)_3C_2F_5$ fulvestrant **2**
$R_1 = (CH_2)_9S(O)(CH_2)_3C_2F_5$, $R_2 = H$ **3**
$R_1 = (CH_2)_3(CF_2)_4(CH_2)_2S(O)(CH_2)_3C_2F_5$, $R_2 = H$ **4**

Synthesis

Synthesis of the 11β Isomer 3 of Fulvestrant. Our synthesis of the 11β isomer of fulvestrant **3**[9] started with the conversion of known 11β-allylestradiol dibenzyl ether **5**[10] to aldehyde **6** by a classical hydroboration[10b]−Swern oxidation sequence (Scheme 1). Elongation of the alkyl side chain was effected following Wittig's protocol in the presence of protected phosphonium salt **10**,[11] leading to olefin **7**. Removal of *tert*-butyldimethylsilyl group by fluoride anion followed by activation of the resulting alcohol function to a mesylate enabled the completion of the side chain by nucleophilic substitution with the sodium salt of 4,4,5,5,5-pentafluoropentylthiol.[12] After selective oxidation of the resulting thioether to sulfoxide **8**,[13] simultaneous benzyl group deprotection and olefin reduction were planned by catalytic hydrogenation. The use of a "10 mol % Pd/C−catalytic Raney nickel" mixture resulted in partial C3-debenzylation and olefin hydrogenation to deliver advanced intermediate **9** in 71% yield. Alternatively, stoichiometric

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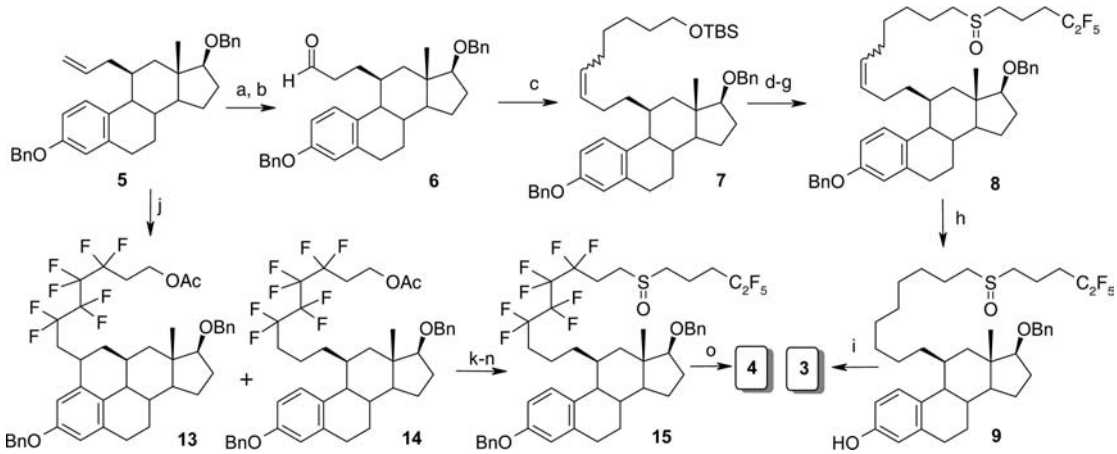
Scheme 1. Synthesis of the 11β Analogues **3** and **4** of Fulvestrant[a]



[a] Reagents and conditions: (a) catechol borane, $LiBH_4$, THF, then H_2O_2, NaOH, EtOH, 0 °C, 6 h, 78%; (b) oxalyl chloride, DMSO, NEt_3, DCM, −78 °C, 87%; (c) $TBSO(CH_2)_6P^+Ph_3$, Br^-, **10**, BuLi, THF, −60 °C, 94%; (d) TBAF, THF, 86%; (e) MsCl, NEt_3, DCM, 91%; (f) $C_2F_5(CH_2)_3SH$, NaH, THF, 0 °C, 12 h, 97%; (g) H_2O_2, HFIP, 0 °C, 5 min, 100%; (h) $Pd(OH)_2$ (100 mol %), H_2 (1 bar), AcOEt, 48 h, 68% (+10% **3**); (i) BCl_3, DCM, −78 °C, 5 min, 56%; (j) **11**, dichloro-1,2-ethane, BEt_3 (4 × 7 mol %), 2 h, then Ph_3SnH (2.5 equiv), dichloro-1,2-ethane, BEt_3 (2 × 10 mol %), 33% (**14**), 15% (**13**); (k) $LiAlH_4$, THF, 98%; (l) MsCl, NEt_3, DCM, 0 °C to room temp, 95%; (m) $C_2F_5(CH_2)_3SH$, NaH, THF, 0 °C, 12 h, 92%; (n) H_2O_2, HFIP, 0 °C, 5 min, 100%; (o) BCl_3, DCM, −78 °C, 10 min, 35%.

Pearlman catalyst $Pd(OH)_2$ afforded monobenzylated compound **9** in acceptable yield (68%) along with some fully deprotected **3** (10% yield). Removal of the retained C17-benzyl group of **9** was finally achieved upon brief exposure to boron trichloride, thus concluding our synthesis of **3** in nine steps and 22% overall yield from the protected 11β-allylestradiol **5**.

Introduction of a Fluorinated Insert in the 11β Isomer 3 of Fulvestrant. On the basis of previous studies,[8] at least an ethylene spacer was required between the steroid core and the perfluorinated fragment to maintain a satisfactory affinity for ERα. Additionally, the sulfoxide moiety had to be remote enough from the perfluorinated fragment to avoid undesired electronic perturbations.[14] On these grounds we selected the iodide $I(CF_2)_4CH_2CHIOAc$ (**11**), which was obtained via selective radical monoaddition of octafluoro-1,4-diiodobutane on vinyl acetate in 58% yield.[15] Sequential addition of **11** and triethylborane radical initiator to a 1,2-dichloroethane solution of the steroid **5** (Scheme 1) followed by a "one pot" radical reduction with triphenyltin hydride delivered adduct **14** in 33% yield along with the pentacyclic steroid derivative **13**.[16] As for the nonfluorinated analogue **3**, further functional readjustments proceeded uneventfully to yield sulfoxide **15**. Final removal of the benzyl protecting groups in **15** could be ultimately effected by brief contact with boron trichloride. The fluorinated estradiol **4** was thus prepared in ∼10% yield after six steps starting from allylestradiol **5**.

Biological Results

Concentrations of hormone and experimental compounds were selected to give unequivocal results in the different experiments.

Interactions of Compounds with Purified hERα Recombinant. When tested for their ability to compete with $[^3H]E_2$ for binding to hERα at 0 °C, fulvestrant (**2**) and its 11β isomer **3** gave similar inhibition curves (relative binding affinity,[14] **2** RBA = 10, **3** RBA = 6). This clearly showed that grafting a functionalized side chain in position 7α or 11β leads to the same result in terms of ligand binding affinity. Furthermore, the affinity toward ERα was not drastically affected by the perfluorination of the side chain of **3** (**4** RBA = 2).

Compounds **2**, **3**, and **4** abrogated the ability of hERα to bind to an LxxLL-coated plate with an almost equivalent potency (Figure S1, Supporting Information), suggesting that they similarly antagonize the recruitment of LxxLL-box coactivators. They also suppressed the E_2-induced increase of ERα binding to such a plate; **4** appeared slightly less efficient than **2** and **3** in this regard, suggesting a small decrease of antiestrogenicity.

Interactions of Compounds with ERα and Antiproliferative Activity in MCF-7 Cells. The 45 min incubations of MCF-7 cells with $[^3H]E_2$ (1 nM) in the absence (control) or presence of increasing amounts of **2**, **3**, or **4** (1 nM to 1 μM) (whole cell competition binding assays) confirmed the ability of the latter to interact with ERα in vivo (Figure S2, Supporting Information). Fulvestrant **2** and its 11β isomer **3** both decreased the capacity of the cells to accumulate $[^3H]E_2$ at concentrations 40- to 50-fold higher than unlabeled E_2, the latter being taken as a reference. The perfluorinated analogue **4** exhibited a similar effect, albeit at a higher concentration (125-fold), suggesting a lower capacity to antagonize E_2 binding.

Assessment of the influence of investigated compounds on MCF-7 cell growth confirmed data generated by whole cell competition binding assays. They abrogated the growth stimulatory effect of E_2 as shown by Figure S3 (Supporting Information). Fulvestrant **2** displayed a somewhat higher antiproliferative activity than its 11β isomer **3** (see values at 10 nM, in the presence or absence of 0.1 nM E_2), although it shared an almost equivalent capacity to antagonize the cellular accumulation of $[^3H]E_2$. Various explanations (duration of drug exposure, interaction with lipophilic molecules, drug metabolization) may be put forward to explain this peculiarity. As expected, the 11β fluorinated E_2 derivative **4** displayed the weakest antagonistic potency. Of note, all compounds decreased cell proliferation even in the absence of E_2, indicating that they also antagonized estrogen independent activation of ERα (cross-talk mechanisms).

Influence of Compounds on ERα Level. At concentrations abrogating cell growth, **2** and its 11β isomer **3** down-regulated ERα, suggesting that they induced its proteosomal degradation (Figure 1). This phenomenon, revealed by Western blotting on cell extracts (Figure 1A) and by immunofluorescence micros-

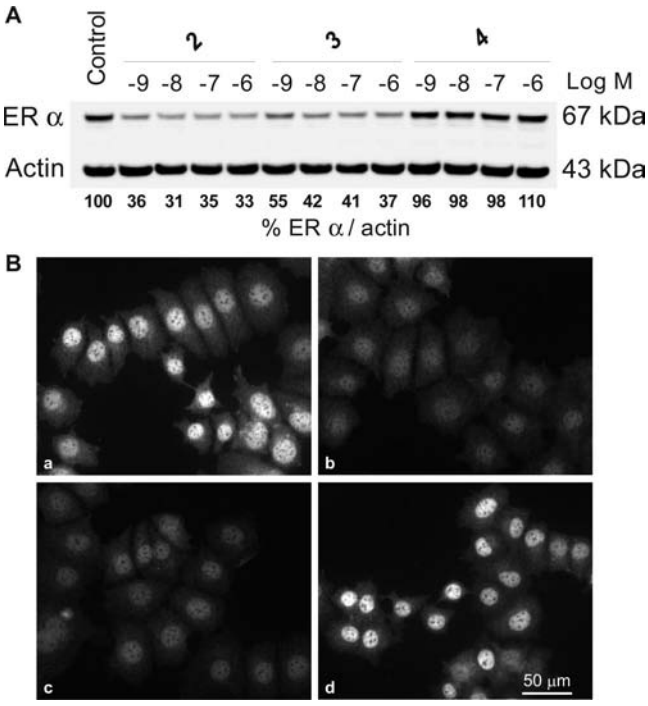


Figure 1. Effect of **2**−**4** on ER level. (A) Western blot analysis. MCF-7 cells were incubated with increasing amounts of each compound for 24 h. Control untreated cells were maintained in culture in parallel. Equal amounts of solubilized proteins were then subjected to Western blotting. Data refer to an experiment performed twice. (B) Immunofluorescence microscopy. The cells were treated for 24 h with **2**, **3**, or **4**, all at 100 nM. ERα was demonstrated by immunofluorescence staining with HC-20 antiserum, as detailed in Experimental Section: (a) untreated cell culture (control); (b) **2**; (c) **3**; (d) **4**; Texas Red labeling.

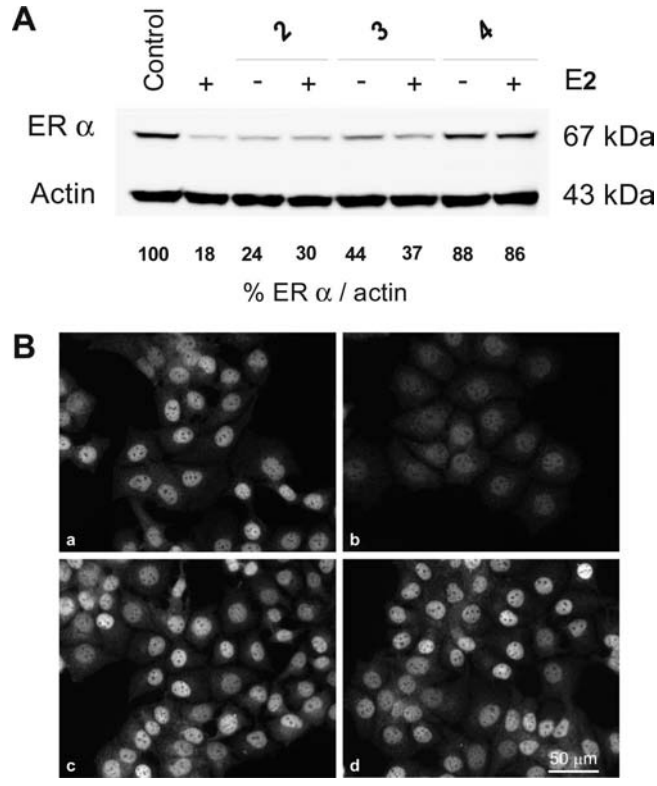


Figure 2. Ability of **2**−**4** to suppress E_2-induced down-regulation of ERα. (A) Western blot analysis. MCF-7 cells were incubated with each compound at 1 μM, alone or in the presence of 10 nM E_2 (a hormone concentration known to induce a demonstrable down-regulation of ERα) for 24 h. Equal amounts of solubilized proteins were then subjected to Western blotting. Data refer to an experiment performed twice. (B) Immunofluorescence microscopy. The cells were treated for 24 h with E_2 at 1 nM and/or **4** at 1 μM (a concentration selected to produce a clear-cut effect on E_2-induced down-regulation of ERα). ERα was demonstrated by immunofluorescence staining with HC-20 antiserum, as detailed in Experimental Section: (a) untreated cell culture (control); (b) E_2; (c) **4**; (d) E_2 and **4** in combination; Texas Red labeling.

copy (Figure 1B) after 24 h of cell exposure to these compounds, was not observed with the perfluorinated derivative **4**.

Interestingly, **4** was able to suppress ERα down-regulation induced by E_2 (Figure 2). It also antagonized the receptor elimination caused by fulvestrant and its 11β isomer (Figure S4, Supporting Information), indicating that the antiproliferative activity of the latter compounds was not strictly related to their capacity to influence the receptor turnover rate.

Discussion

Fulvestrant, as well as some other steroidal antiestrogens (i.e., ICI 164,384, RU 58668), is totally devoid of estrogenicity ("pure antiestrogen"). This has been attributed to the fact that these antagonists enhance ERα proteasomal degradation in estrogen responsive cells.[3,17,18] Such considerations have given rise to the concept of selective estrogen receptor down-regulators (SERDs),[19] defining a pharmacological group of estrogen antagonists distinct from selective estrogen receptor modulators (SERMs) ("partial antiestrogens") which stabilize ERα.[3,20] Yet our study reveals that fluorination of the alkyl side chain in an 11β isomer **3** of fulvestrant (i.e., **4**), while not affecting antiestrogenicity, suppresses the capacity to down-regulate ERα in breast carcinoma cells.

The response of estrogen target cells to hormonal stimulation is largely controlled by ligand-induced modulation of ERα level.[21] Previous work[22] has unraveled molecular events underlying ERα degradation induced by E_2 or fulvestrant. Binding of these ligands promotes the association of ERα with Uba3/APP-BP1, the activating enzyme complex for NEDD8. This leads to the neddylation and activation of a cullin-based ubiquitin ligase (E3). E3, along with an ubiquitin-conjugating enzyme (E2), catalyzes the ubiquitination of ERα. Finally, ubiquitinated ERα is degraded in the proteasome compartment. According to a recent study,[23] two lysines (K302 and K303) located in the hinge region of ERα play a pivotal role in the above-described process, since they seem to be targets for polyubiquitination. In the context of the current study, it is reasonable to assume that the perfluorinated side chain of **4** somehow interferes with ubiquitin−proteasome-mediated breakdown of ERα by preventing E3 neddylation or ERα ubiquitination. Yet it is noted that this occurs without a loss of antiestrogenicity. As far as the antagonist profile of **4** is concerned, it still remains to be determined whether it is a pure antiestrogen despite the fact that it has lost the ability to provoke ERα down-regulation. Although the issue is beyond the scope of the present study, it is of importance, since the properties of **4** suggest that pure antiestrogenicity is not necessarily associated with ERα down-regulation.

As shown by the X-ray crystallographic analysis of the LBD of an estrogen receptor bound to a steroidal antiestrogen (ICI 164,384 complexed with the LBD of ERβ), the side chain of the ligand protrudes from the binding cavity, preventing helix 12 stabilization over the latter and the subsequent recruitment of LxxLL motif-containing coactivators.[2] The finding that the 11β isomer of fulvestrant (**3**) antagonizes coactivator binding with a similar efficiency is not surprising in view of the

equivalence of the 7α and 11β substitutions in terms of interactions with ERα.[6,7] The side chain of these two isomers probably protrudes from the LBD in a similar way, resulting in interactions with a same region of the receptor surface. Partial fluorination of this side chain, which limits its flexibility, would logically decrease such an interaction. Could this property be related to the absence of effect of **4** on ERα turnover? Analysis of various 11β perfluorinated estrogens and antiestrogens may be informative in this respect. Whatever the answer would be, our data suggest that an interaction with a given part of the receptor surface is not required to abrogate the recruitment of coactivators harboring an LxxLL motif. Repulsion of such coregulators from the receptor area seems to be a more likely explanation: the functionalized terminal part of the side chain may shield the receptor against interacting motifs of coactivators rather than obstruct their binding sites. In fact, this concept is in agreement with previous reports suggesting the existence of a "passive" antagonism in addition to an "active" antagonism based on a direct blockade of the recruitment site.[24] Studies devoted to the effect of flexible and nonflexible side chains at 11β are warranted to test this hypothesis.

Experimental Section

General. Usual workup refers to a quench with saturated NH_4Cl solution, extraction with CH_2Cl_2, washing of the organic layers with water, drying over $MgSO_4$, and concentration under reduced pressure. Additional data may be found in the Supporting Information.

Synthesis of the 11β Analogue of Fulvestrant. 3-(3,17β-Bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)propan-1-al 6. To a solution of oxalyl chloride (296 mg, 2.33 mmol, 1.1 equiv) in dichloromethane (5 mL) was added dimethyl sulfoxide (363 mg, 4.66 mmol, 2.2 equiv) at −60 °C, followed after 5 min by a solution of 3-(3,17β-bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)propan-1-ol[10b] (1.08 g, 2.12 mmol) in dichloromethane (5 mL). After 15 min, triethylamine (1.07 g, 10.6 mmol, 5 equiv) was added dropwise. After the mixture was warmed to room temperature and the usual workup, column chromatography on silica gel (CH_2Cl_2) afforded aldehyde **6** (938 mg, 1.85 mmol, 87%) as a white foam.

[9-(3,17β-Bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)non-6-enyloxy]-*tert*-butyldimethylsilane 7. A solution of *n*-butyllithium (2.5 M in hexanes, 570 μL, 1.43 mmol, 1.9 equiv) was slowly added to a suspension of the phosphonium salt 10 (817 mg, 1.5 mmol, 2 equiv) in THF (3 mL) at −78 °C. After the mixture was stirred for 15 min, a solution of aldehyde **6** (380 mg, 0.75 mmol) in THF (3 mL) was added. After 30 min of being stirred, the mixture was warmed to room temperature for an additional 2 h. After usual workup, the silylated product **7** (499 mg, 94% yield) was obtained by flash chromatography on silica gel (pentane/CH_2Cl_2, 3/2) as a glass.

3,17β-Bisbenzyloxy-11β-[9-(4,4,5,5,5-pentafluoropentane-1-sulfinyl)non-3-enyl]estra-1,3,5(10)-triene 8. To a solution of steroid **7** (480 mg, 0.68 mmol) in dry THF (3 mL) was added solid tetrabutylammonium fluoride (530 mg, 2.04 mmol, 3 equiv) at 0 °C. The reaction was stopped after completion (TLC) by pouring it into water (2 mL) and CH_2Cl_2 (10 mL). After usual workup, column chromatography (CH_2Cl_2/MeOH, 99.5/0.5) afforded the free alcohol 9-(3,17β-bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)non-6-en-1-ol (344 mg, 86%) as a glass. Triethylamine (86 mg, 0.86 mmol, 1.5 equiv) and mesyl chloride (78 mg, 0.68 mmol, 1.2 equiv) were added at 0 °C to a solution of the preceding alcohol (340 mg, 0.57 mmol) in CH_2Cl_2 (4 mL). After usual workup, the crude mixture was purified by column chromatography (CH_2Cl_2) to afford methanesulfonic acid 9-(3,17β-bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)non-6-enyl ester (346 mg, 91%) as a white solid. To a solution of the preceding mesylate (55 mg, 0.082 mmol) in distilled THF (0.5 mL) was first added 4,4,5,5,5 pentafluoropentylthiol (32 mg, 0.164 mmol, 2 equiv) and then sodium hydride (80%, 4.5 mg, 0.147 mmol, 1.8 equiv). After usual workup, the crude mixture was purified by column chromatography on silica gel (pentane/CH_2Cl_2, 2/3) to yield 3,17β-bisbenzyloxy-11β-[9-(4,4,5,5,5-pentafluoropentylsulfanyl)non-3-enyl]estra-1,3,5(10)-triene (61 mg, 97%) as a glass. Hydrogen peroxide (35% solution, 31 μL, 0.32 mmol, 2 equiv) was slowly poured at 0 °C into a solution of the preceding thioether (120 mg, 0.16 mmol) in hexafluoroisopropanol (0.6 mL). After 5 min of stirring, sodium sulfite (25 mg, 0.2 mmol) was added and the mixture was stirred for 30 min. Usual workup afforded, without further purification, the desired sulfoxide **8** in quantitative yield (121 mg).

3-Hydroxy-11β-[9-(4,4,5,5,5-pentafluoropentane-1-sulfinyl)non-3-enyl]-17β-benzyloxyestra-1,3,5(10)-triene 9. Palladium(II) hydroxide (100% mass) was added to a solution of sulfoxide **8** (65 mg, 0.083 mmol) in ethyl acetate (2 mL). After 24 h of being stirred under hydrogen, the crude mixture was filtered through a pad of Celite and purified by preparative TLC (CH_2Cl_2/MeOH, 95/5) to afford the monodebenzylated product **9** as a glass. Yield 68%.

3,17β-Dihydroxy-11β-[9-(4,4,5,5,5-pentafluoropentane-1-sulfinyl)nonyl]estra-1,3,5(10)-triene 3. Steroid **9** (35 mg, 0.045 mmol) was dissolved in CH_2Cl_2 (1.25 mL), and the solution was cooled to −78 °C. A solution of BCl_3 in CH_2Cl_2 (0.5 M, 0.6 mL, 0.3 mmol, 6.6 equiv) was then slowly added. After 10 min, the reaction was quenched with methanol (1 mL). The final compound was obtained after usual workup by purification on preparative TLC (CH_2Cl_2/MeOH:90/10) as a white solid. Yield 56%.

Synthesis of the Fluorinated 11β Analogue of Fulvestrant 4. Acetic Acid 3,3,4,4,5,5,6,6-Octafluoro-1,6-diiodohexyl Ester 11. To a solution of 1,4-diiodoperfluorobutane (4.85 g, 10.7 mmol) in 1,2-dichloroethane (15 mL) was added freshly distilled vinyl acetate (0.4 mL, 4.28 mmol, 0.4 equiv) and 1 M triethylborane (0.2 mL, 0.2 mmol, 1.8 mol%). This addition was repeated two times after a 30 min delay. The solvent was removed under vacuum after 1 h, and the crude mixture was purified by silica gel chromatography (CH_2Cl_2/pentane, 40/60). Monoadduct **11** (2.82 g, 58%) was isolated as a colorless oil (highly unstable when exposed to light).

Acetic Acid 9-[3,17β-Bisbenzyloxyestra-1,3,5(10)-trien-11β-yl]-3,3,4,4,5,5,6,6-octafluorononyl Ester 14. To a solution of 11β-allylestrane **5** (680 mg, 1.38 mmol) in 1,2-dichloroethane (2.15 mL) were simultaneously added adduct **11** (4 × 185 mg = 745 mg, 1.38 mmol, 1 equiv) and triethylborane (1 M solution in hexane, ∼4 × 0.10 mL = 0.38 mL, 28% mol) every 30 min and in four equal portions. The mixture was stirred 5 min at room temperature, and triphenyltin hydride (∼2 × 0.6 g = 1.21 g, 3.45 mmol, 2.5 equiv) and triethylborane (0.28 mL, 2 × 10% mol) were introduced in two equal portions. Reaction was allowed to proceed overnight. After usual workup, column chromatography on silica gel (pure pentane to CH_2Cl_2/pentane, 30/70) afforded addition product **14** (354 mg, 33%) as a glass. Acetic acid 7-[1,11β-ethano-3,17β-bisbenzyloxyestra-1,3,5(10)-trien-1′-yl]-3,3,4,4,5,5,6,6-octafluorononyl ester **13** was also isolated (15%).

3,17β-Bisbenzyloxy-11β-[4,4,5,5,6,6,7,7-octafluoro-9-(4,4,5,5,5-pentafluoropentane-1-sulfinyl)nonyl]estra-1,3,5(10)-triene 16. The preparation of this compound closely follows that of its nonfluorinated analogue **8** (vide supra). $LiAlH_4$ (10 mg, 0.262 mmol, 1.5 equiv) was added to a solution of acetate **14** (135 mg, 0.173 mmol) in THF (4.5 mL). After completion of the reaction, water (10 μL), a 15% NaOH solution (10 μL), and finally water (30 μL) were added. The precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to obtain 9-(3,17β-bisbenzyloxyestra-1,3,5(10)-trien-11β-yl)-3,3,4,4,5,5,6,6-octafluorononan-1-ol (132 mg, 98%) as a glass. To a solution of the preceding alcohol (43 mg, 0.058 mmol) in CH_2Cl_2 (1 mL) were added triethylamine (17 mg, 0.168 mmol, 2.9 equiv) and methanesulfonyl chloride (16 mg, 0.140 mmol, 2.4 equiv). After usual workup, the crude mixture was purified by column chromatography (CH_2Cl_2) to afford methanesulfonic acid 9-[3,17β-bisbenzyloxyestra-1,3,5(10)-trien-11β-yl]-3,3,4,4,5,5,6,6-octafluorononyl ester (45 mg, 95%) as a glass. To a solution of the preceding mesylate (85 mg, 0.10 mmol) and 4,4,5,5,5-pentafluoropentylthiol (100 mg,

0.51 mg, 5 equiv) in THF was added NaH (80%, 12 mg, 0.4 mmol, 4 equiv) at 0 °C. The reaction was allowed to proceed overnight. After usual workup, column chromatography (CH_2Cl_2/pentane, 75:25) afforded 3,17β-bisbenzyloxy-11β-[4,4,5,5,6,6,7,7-octafluoro-9-(4,4,5,5,5-pentafluoropentylsulfanyl)nonyl]estra-1,3,5(10)-triene (88 mg, 92%) as a glass. Oxidation of this sulfide, as described above for the preparation of **8**, afforded the title compound yield 100%.

3,17β-Dihydroxy-11β-[4,4,5,5,6,6,7,7-octafluoro-9-(4,4,5,5,5-pentafluoropentane-1-sulfinyl)nonyl]estra-1,3,5(10)-triene 4. Brief treatment of dibenzylether **16** by boron trichloride in CH_2Cl_2 as described above for the preparation of **3** gave the title compound **4**. Yield 35%.

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Supporting Information Available: Biological procedures, biological properties of **2**−**4**, and spectral and analytical data for **3**−**16** and all intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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